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Neurochemical Mechanisms Mediating Recovery of Function

Annual Report

David Olton, Gary Wenk, Zoltan Annau

November 1984

Supported by:

U.S. ARMY MEDICAL RESEARCH AND DEVELOPMENT COMMAND
Fort Detrick, Frederick, Maryland 21701-5012

Contract No. DAMD 17-82-C-2225

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The Johns Hopkins University
Baltimore, Maryland 21218

SECURITY CLASSIFICATION OF THIS PAGE

REPORT DOCUMENTATION PAGE

Form Approved OMB No. 0704-0188

1a. REPORT SECURITY CLASSIFICATION: Unclassified	1b. RESTRICTIVE MARKINGS
2a. SECURITY CLASSIFICATION AUTHORITY	3. DISTRIBUTION/AVAILABILITY OF REPORT: Approved for public release; distribution unlimited
2b. DECLASSIFICATION/DOWNGRADING SCHEDULE	
4. PERFORMING ORGANIZATION REPORT NUMBER(S)	5. MONITORING ORGANIZATION REPORT NUMBER(S)
6a. NAME OF PERFORMING ORGANIZATION: Johns Hopkins Univ. Psychology Dept. / 6b. OFFICE SYMBOL (If applicable)	7a. NAME OF MONITORING ORGANIZATION
6c. ADDRESS (City, State, and ZIP Code): Baltimore, MD 21218	7b. ADDRESS (City, State, and ZIP Code)
8a. NAME OF FUNDING/SPONSORING ORGANIZATION: U.S. Army Medical Research & Development Command / 8b. OFFICE SYMBOL (If applicable)	9. PROCUREMENT INSTRUMENT IDENTIFICATION NUMBER: DAMD 17-82-C2225
8c. ADDRESS (City, State, and ZIP Code): Fort Detrick, Frederick, Maryland 21701-5012	10. SOURCE OF FUNDING NUMBERS

PROGRAM ELEMENT NO.	PROJECT NO.	TASK NO.	WORK UNIT ACCESSION NO.
61102A	61102BS11	EE	010

11. TITLE (Include Security Classification): Neurochemical mechanisms mediating recovery of function.

12. PERSONAL AUTHOR(S): David Olton, Gary Wenk, Zoltan Annau

13a. TYPE OF REPORT	13b. TIME COVERED	14. DATE OF REPORT (Year, Month, Day)	15. PAGE COUNT
Annual Report	FROM 8/1/83 TO 7/31/84	1984 November	37

16. SUPPLEMENTARY NOTATION

17. COSATI CODES			18. SUBJECT TERMS (Continue on reverse if necessary and identify by block number)
FIELD	GROUP	SUB-GROUP	cholinergic lesions, ibotenic acid, rats, neurochemical/ behavioral changes
06	15		
06	16		

19. ABSTRACT (Continue on reverse if necessary and identify by block number)

See reverse side.

20. DISTRIBUTION/AVAILABILITY OF ABSTRACT	21. ABSTRACT SECURITY CLASSIFICATION
☐ UNCLASSIFIED/UNLIMITED ☒ SAME AS RPT. ☐ DTIC USERS	Unclassified

22a. NAME OF RESPONSIBLE INDIVIDUAL	22b TELEPHONE (Include Area Code)	22c. OFFICE SYMBOL
Mrs. Virginia Miller	301/663-7325	SGRD-RMS

DD Form 1473, JUN 86 — Previous editions are obsolete. — SECURITY CLASSIFICATION OF THIS PAGE

BLOCK 19 ABSTRACT

We investigated whether the type of motivation, reinforcement, response-reinforcement contingency, or the response itself was influenced by ibotenic acid lesions in the basal forebrain. The tasks were postoperative acquisition of a win-stay spatial discrimination in a T-maze, a win-shift spatial discrimination on a radial arm maze, active avoidance in a shuttle box, and passive avoidance in a shuttle box. Rats with lesions had significantly impaired choice accuracy in both maze tasks, took significantly fewer trials to reach criterion in the active avoidance task, and took significantly more trials to reach criterion in the passive avoidance task. These results indicated that equivalent behavioral changes are obtained from lesions in the nucleus basalis magnocellularis and the medial septal area in tasks that vary in motivation and reinforcement contingencies.

Investigations of changes in various biochemical parameters (choline acetyltransferase, serotonin receptor binding, biogenic amine and metabolite levels in cortex, caudate and hippocampus) indicate that the ibotenic acid lesions are selective for the cholinergic system when used in the described manner.

Microinfusion experiments indicate that enkephalinergic, GABAergic, and possibly glutamatergic receptors are located on basal forebrain cholinergic neurons. This information provides insight into the nature of neuronal afferents that influence the activity of this cholinergic system, and should help determine the function of this system.

We have begun to understand the importance of the basal forebrain cholinergic system for memory and cognition and have preliminary data on how to produce a recovery of function following exposure to toxins which damage this brain region.

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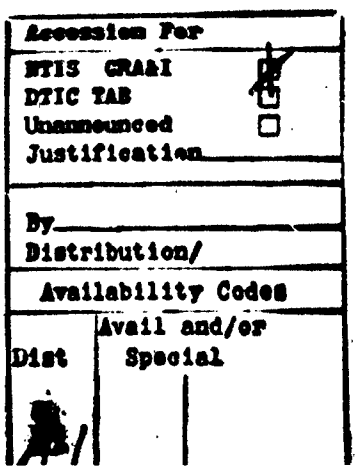

SUMMARY

This annual report discusses our accomplishments during the previous 2 years, outlines our approach for the third year of the contract and discusses briefly some of the experiments we are now undertaking.

The first annual report outlined the initial goals: 1) to describe the biochemical and morphological changes that occur in the neocortex and hippocampus following destruction of neurons in the basal forebrain produced by injection of ibotenic acid and 2) to determine the influence of such lesions upon behavior, particularly upon the ability of lesioned animals to perform tasks which require trial-dependent and trial-independent memory. We are now able to produce reliably and accurately lesions in the basal forebrain cholinergic system in order to study changes in biochemistry and behavior.

We investigated whether the type of motivation, reinforcement, response-reinforcement contingency, or the response itself was influenced by lesions in the basal forebrain. The tasks were postoperative acquisition of a win-stay spatial discrimination in a T-maze, a win-shift spatial discrimination on a radial arm maze, active avoidance in a shuttle box, and passive avoidance in a shuttle box. Rats with lesions had significantly impaired choice accuracy in both maze tasks, took significantly fewer trials to reach criterion in the active-avoidance task, and took significantly more trials to reach criterion in the passive avoidance task. These results indicated that equivalent behavioral changes were obtained from lesions in the nucleus basalis magnocellularis (NBM) and the medial septal area (MSA) in tasks that vary in motivation and reinforcement contingencies. The results of these studies are consistent with earlier reports that both the NBM and the MSA may be involved in memory processes.

Experiments were also undertaken to determine whether 1) basal forebrain lesions impaired trial-dependent or trial-independent memory selectively, 2) these types of memories could be dissociated within a task by the lesions, or 3) task difficulty is the sole factor determining whether basal forebrain lesions produced behavioral impairments. As described in the first annual report, a difficult task with a long delay interval produced performance impairments in rats with lesions, while an easier task with a short delay did not. We now also report that only trial-dependent memory was impaired by basal forebrain lesions.

The development of these behavioral tests for rats with lesions provides an animal model that has allowed us to make progress in understanding how impairments can be treated with pharmacological agents. The details of these studies will be presented in the third annual report. Briefly, we have shown that certain compounds, used in an appropriate manner, are able to accelerate the natural recovery processes. These agents hold significant potential as a possible therapy following basal

forebrain cholinergic destruction.

We have also investigated a novel compound known as BM-5. This agent is a postsynaptic muscarinic agonist and a presynaptic muscarinic antagonist. It was expected to accentuate steady-state activity in the cholinergic system. We discovered that treatment with this compound had no affect on rats' behavioral performance; it actually may have impaired the performance of certain rats. We have discontinued our studies on this agent.

We now report on data that indicate that enkephalinergic, GABAergic, and possibly glutamatergic receptors are located on basal forebrain cholinergic neurons. This information provides insight into the nature of neuronal afferents that influence the activity of this cholinergic system, and should help determine the function of this system.

During the third contract year, we intend to continue our investigation of other pharmacological compounds in various combinations, and their ability to produce recovery of lost behavioral functions. We also intend to introduce new behavioral tasks. These studies will investigate the influence of basal for brain lesions on the ability of rats to learn odor discriminations and to judge accurately time intervals between conditioned stimuli and reinforcement. We also intend to examine further the long-term morphological changes that occur following the production of basal forebrain lesions in rats. We will continue to investigate biochemical changes in non-cholinergic systems in the hippocampus and cortex. We currently are examining acute and chronic changes in serotonergic receptor density and endogenous levels of serotonin, and its major metabolite (5-hyroxyindole acetic acid), following the production of basal forebrain lesions in rats.

In summary, we have met the goals that were outlined for the second year, and have successfully completed some critical experiments necessary to determine whether we can produce a recovery of function using selected pharmacotherapies. We have begun to understand the importance of the basal forebrain cholinergic system for memory and cognition and have preliminary data on how to produce a recovery of function following exposure to toxins which damage this brain region. Given our progress during the first 2 years, we are optimistic about the prospects for continued success during the third and final year of this contract.

FOREWORD

In conducting the research described in this report, the investigators adhered to the "Guide for the Care and Use of Laboratory Animals," prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council (DHEW Publication No. (NIH) 78-23, Revised 1978).

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STATEMENT OF PROBLEM

The research described herein investigated the neurochemical and behavioral changes subsequent to the selective injury to the cholinergic neurotransmitter system in the basal forebrain of the rat. An understanding of the neurochemical and behavioral sequelae following exposure to a cholinergic toxin, such as soman, is essential for the successful design of an effective pharmacotherapy.

BACKGROUND AND RATIONALE

Within the basal forebrain of the rat is a core of acetylcholinergic (Ch) neurons (1) that has been divided into several regions which include the nucleus basalis magnocellularis (NBM) and diagonal band of Broca/medial septal area complex (MSA) (2). The NBM projects Ch afferents primarily to neocortex, and the MSA projects Ch afferents primarily to the hippocampus.

Ibotenic acid (IBO) was used to make lesions in the NBM and the MSA. IBO destroys neuronal perikayra at the site of the injection, but spares fibers of passage and does not damage neurons at a distance (3). Consequently, the behavioral effects following these lesions are due to specific damage to these cells, rather than to more generalized damage to neuronal pathways through the area.

The behavioral tasks examined in these studies were chosen so that the pattern of results will extend the conclusions drawn from previous experiments reported in the literature and from our laboratory and will provide a more complete description of the behavioral changes produced by these lesions (4). The tasks were postoperative acquisition of: a win-stay spatial discrimination in a T-maze, a win-shift spatial discrimination on a radial arm maze, active avoidance in a shuttle box, passive avoidance in a shuttle box, and a 14-item list length in a Stone maze.

Performance in many of these tasks is affected by lesions of the hippocampus and anticholinergic drugs (5). If the afferents to the hippocampus from the MSA are also involved in these behaviors, selective damage to the cells of the MSA by IBO ought to produce a similar pattern of results.

The frontal neocortex and the hippocampus, projection areas of the NBM and MSA, respectively, have been described as components of a system involved in memory (6). Consequently, lesions were also made in both the NBM and the MSA with the expectation that the behavioral effects of these lesions would be similar. Because parametric manipulations of behavioral variables can influence the magnitude of the behavioral change produced by a lesion, the inclusion of both types of lesions in the same experiment is important to provide an accurate comparison of their behavioral effects.

In the three maze tasks, motivation was induced by food deprivation and the reinforcement was food. In the two

shuttle-box tasks, motivation was induced by shock and the reinforcement was avoidance of and escape from shock. The maze tasks differed in their response-reinforcement contingencies. In the T-maze, the correct response was returning to the arm previously visited in that trial (win-stay, match-to-sample strategy). In the radial arm maze, the correct response was going to an arm not previously visited in that trial (win-shift, non-match-to-sample strategy). In the Stone maze, the correct response was made by remembering the sequence of 14 correct turns. The avoidance tasks differed in the type of response required to avoid shock. Active avoidance required response initiation (going across the barrier to the other compartment), while inhibitory avoidance required inhibition of that response (remaining in the same compartment).

METHODS

General

Subjects

Male albino rats (300-350 g) were obtained from Charles River. Each rat was housed individually throughout testing with free access to water, and was maintained on a 16:8 light/dark cycle with lights on at 7:00 A.M. Each rat in the two maze experiments was deprived to 85% of its ad-lib weight prior to shaping and was maintained at this weight, plus 5 g per week for growth, throughout testing. At the completion of the day's testing, each rat was fed the appropriate amount of Charles River Rat Formula. Each rat in the avoidance experiments was fed ad-lib throughout testing.

Ten rats were assigned to each group within the tasks: bilateral lesions in the NBM, bilateral lesions in the MSA, bilateral lesions in both MSA and NBM, and operated control.

Surgery

Each rat received 0.25 cc of 0.5 mg/ml atropine methyl bromide (Sigma, St. Louis, Mo.) intraperitoneally. Thirty minutes later, each rat was anesthesized with 0.3 ml/kg Chloropent (Fort Dodge Laboratories, Fort Dodge, Iowa). The rat was placed in a stereotaxic instrument with the incisor bar set so that bregma and lambda were in the same horizontal plane. The scalp was incised and retracted. Holes were drilled through the skull in the appropriate locations. If the rat was to receive a lesion, IBO was injected in the appropriate location. The scalp was sutured and the rat was removed from the stereotaxic instrument and placed under a heat lamp until it awakened.

Coordinates for the NBM lesions were 0.8 mm posterior to bregma, 2.6 mm lateral to the central sinus, and 6.9 mm ventral from dura. Lesions were produced by injecting 25 nmole of IBO (Sigma, St. Louis, Mo.) in 1.0 ul of phosphate-buffered saline

(pH 7.4) with a 1.0 ul Hamilton syringe during 10 min. The syringe was left in place for 5 minutes after the completion of the infusion. Coordinates for the MSA lesions were: 0.8 mm anterior tc bregma, on the midline, and 5.8 mm ventral from dura. Lesions were produced by injecting 18 nmole of IBO in 600 nl of phosphate-buffered saline during a period of 5 min. Control rats received the same surgical treatment as the lesioned rats except that no IBO was injected.

Biochemical Analyses

At the completion of behavioral testing, each rat was decapitated and the brain rapidly removed and cooled on ice.

Choline Acetyltransferase. The decrease in endogenous levels of choline acetyltransferase (ChAT) activity indicated the effectiveness of the lesions. Tissue samples (100 mg) were taken from frontolateral cortex (areas 2 and 10, according to the atlas of Krieg [7]), and the entire hippocampus. ChAT activities were determined using the method of Fonnum (8) to ascertain the effectiveness of the lesions. Protein was measured according to Lowry et al. (9).

Biogenic Amines. In order to determine the effects of the lesions on noncholinergic systems, endogenous levels of the neurotransmitter biogenic amines and of their metabolites were measured. Tissues samples from the cortex, hippocampus and caudate nucleus of rats with NBM lesions were also assayed for changes in biogenic amine levels. Sections were homogenized in perchloric acid (0.05N) and centrifuged at 30,000 x g for 15 min. An aliquot of the supernatant was filtered and injected directly into the high performance liquid chromatography (HPLC) system. The HPLC system, detector, and chromatographic conditions have been described previously (10). Endogenous levels of norepinephrine, dopamine, dihydroxyphenalacetic acid, homovanilic acid, 5-hydroxyindolacetic acid, and serotonin were determined.

2-Deoxyglucose Uptake. As a measure of glucose utilization and energy metabolism, 2-deoxyglucose uptake was determined. Five rats were given bilateral lesions in the MSA and NBM using IBO. Three weeks later, these rats were prepared with prophemeral artery cannulae and examined for 2-deoxyglucose uptake. Local cerebral glucose utilization, expressed in umoles per 100g per min, was examined in 22 different brain regions in five rats (11). The regions included dorsal and ventral hippocampus, subiculum, thalamic nuclei and all regions and layers of cortex.

Serotonin Receptor Binding. An understanding of the nature of receptors that exist on Ch neurons in the basal forebrain is needed in order to design a possible therapy for recovery of function. Neurons which survive a lesion might be stimulated to

alter their activity using pharmacological agents selected for receptors that are present on these neurons. Sixteen rats were prepared with unilateral NBM lesions using IBO as described previously. Two weeks following the production of these lesions, the rats were sacrificed by decapitation. The substantia innominata and ventral globus pallidus region and the frontal neocortex were isolated and stored frozen for further analysis. The neocortex was examined for ChAT activity and the substantia innominata and ventral globus pallidus complex was examined for the level of serotonin receptor density according to the method of Leysen et al.(11b). Decreases in ChAT activity in the cortex due to the loss of cholinergic cells in the NBM were correlated with changes in the density of serotonin receptors in the NBM.

Histology

After removal of selected sections for biochemical analyses, the remaining brain tissue was fixed in a 10% formalin:30% sucrose solution. The brain was frozen and sectioned coronally at 30 um with a frozen stage microtome. Every fifth section throughout the lesion site was mounted on a glass slide and stained with cresyl violet. The size and location of the lesions were determined by microscopic examination for loss of magnocellular neurons and the presence of gliosis.

Statistics

Unless specifically stated, the data were analyzed using an analysis of variance with post hoc Sheffe contrasts.

T-Maze Discrimination

Apparatus

The apparatus was a wooden T-maze. The stem was 33 cm long and 9 cm wide; each arm was 49 cm long and 9 cm wide. On each side of the stem and arms was an edge 5 cm high. The maze was elevated 22 cm above the surface of a table. At the end of each arm was a food cup 1 cm in diameter and 1 cm deep.

Procedure

After recovering from surgery, all rats were shaped to go down the arms of the maze for a food reward (Thrive Cat Food, Purina) during a 5 day period. On the first day, food was placed liberally around the maze, and the rat was allowed to explore. On succeeding days, the food was gradually restricted to the ends of the arms.

For testing, each trial consisted of a forced run, in which the rat was directed to one arm, and a choice run, in which the

rat was able to choose freely either arm. For the forced run, one piece of food was placed in the cup at the end of one arm, and a large piece of wood blocked the entrance to the other arm. The rat was placed on the starting platform; the guillotine door was raised, allowing the rat to run down the stem and enter the unblocked arm to get the food. For the choice run, the rat was again placed on the starting platform. One piece of food was placed on the arm to which the rat had been forced, and the block of wood was removed from the other arm. After 5 sec, the guillotine door was raised and the rat was allowed to run down the stem and freely choose one of the arms. A response was recorded when the rat placed its head more than 10 cm down an arm. If the rat chose the arm which it had entered during the preceding forced run, a correct response was recorded and the rat was allowed to eat the food. If the rat chose the arm that had been blocked during the preceding forced run, an incorrect response was recorded. The rat was then returned to its home cage.

Once per day, 5 days each week, each rat was given a test session consisting of 8 trials separated by an intertrial interval of approximately 4 min. Testing was continued until the rat reached a criterion of at least 7 correct responses in the 8 trials of each day for 5 consecutive days.

Radial Arm Maze Discrimination

Apparatus

The apparatus was a radial maze, elevated 1.4 m above the floor. Each of the 8 arms, 97 cm long and 9 cm wide, was connected to an octagonal central platform, 30 cm in diameter. At the distal end of each arm was a hole, 1.5 cm wide and 1.5 cm deep, which served as a food cup. Along the side of each arm was an edge, 4 cm high. Around the outside circumference of the central platform, between the arms, was a barrier 34 cm high. At the end of each arm by the central platform was a guillotine door, 34 cm high, that was attached to strings which were manipulated by the experimenter to open and close the doors.

Procedure

After recovery from surgery, each rat was shaped to go to the ends of the arms for a food reward. During the first day, food was spread throughout the maze. During the subsequent days it was progressively restricted to the ends of the arms until by the fourth day it was only in food cups. Each rat was placed on the maze for 15 min each day.

At the start of each trial, one pellet of food was placed in the food cup at the end of each arm. The rat was placed in the central platform, the guillotine doors were raised, and the rat was allowed to choose among the arms. A response was defined as the rat placing all four legs on an arm. When the rat entered an

arm for the first time during each trial, a correct response was recorded. When the rat returned to an arm previously visited in that trial, an incorrect response was recorded. When the rat returned to the central platform, the guillotine doors were lowered. After 5 sec, all the guillotine doors were again raised. This process continued until the rat had visited each of the 8 arms, has made a total of 16 responses, or until 10 minutes had passed since the start of the trial, whichever came first. Each rat was given 1 trial per day, 5 days each week. Testing continued until the rat met a criterion of at least 8 correct responses in the first 9 responses for 4 consecutive days.

Active and Passive Avoidance Discriminations

Apparatus

The apparatus was a box, 13.0 X 43.0 X 27.0 cm, with the sides and top constructed of clear Plexiglas. The floor consisted of stainless-steel rods, 0.9 cm in diameter, spaced 1.7 cm apart. The box was divided into two compartments by an opaque guillotine door which slid down to the top of a barrier, 4.5 cm high, in the middle. The conditioned stimulus (CS) was 50-decibel white noise and the illumination of a 6-watt light in the compartment occupied by the rat. The unconditioned stimulus (UCS) was a 0.5 ma shock delivered in 0.2 sec pulses by a shock generator (GSC model E1064GS). The CS-UCS interval was 10 sec.

Procedure

Two weeks after surgery, rats were placed 2 at a time in the shuttlebox for 30 minutes. The guillotine door was raised and the rats were allowed to explore freely; neither the CS nor the UCS was present.

Acquisition of 2-way active avoidance was tested the next day. A trial began when the guillotine door was raised and was terminated when the rat left the shock compartment and crossed over the center barrier with all 4 feet. Each rat was given unavoidable shock on the first 2 trials; the guillotine door remained shut and was not raised until the onset of the shock. At the start of each subsequent trial, the CS was presented and the guillotine door was raised. If the rat did not cross over the barrier to the other compartment within 10 sec after the onset of the CS, the UCS was presented until the response was made. After the rat moved to the other compartment, the guillotine door was lowered and the CS and UCS were terminated. The rat remained in the compartment which it had just entered for 30 sec, after which the guillotine door was again raised and the CS presented. A correct response was recorded if the rat crossed over the barrier before the onset of the UCS. An incorrect response was recorded if the rat did not cross over the barrier before the onset of the UCS.

The retention of active avoidance was assessed the next

day. Each rat was given the same procedure, except that no unavoidable shocks were given.

During the next day, each rat received 10 active avoidance trials as during training. Passive avoidance training began on trial 11. At the beginning of each trial, the guillotine door was raised, the CS was presented in the compartment with the rat, and the UCS was presented in the opposite compartment. If the rat made an incorrect response and crossed to the unoccupied compartment, it received shocked. If the rat made a correct response and did not cross over the barrier for 20 seconds, no shock was given. Each rat was trained to a passive and active avoidance criterion of 5 consecutive correct responses.

Stone Maze

Experimental Design

The experiment was performed in 2 replications. The rats in the first replication were tested on the Stone maze. The rats in the second replication were tested on both the Stone maze and the radial arm maze. In each replication the rats were randomly assigned to 1 of 2 groups: Rats in one group (BF) had bilateral lesions of both the NBM and the MSA. Rats in the other group (CON) had control operations.

Apparatus

A wooden runway, used to shape the rats, was 189 cm long, 8 cm wide, with walls 12 cm high.

The Stone maze had walls of Plexiglas, 0.6 cm wide and 42 cm high, with pathways 12 cm wide. The base of the maze was plywood. Sliding plastic panels were placed at 6 choice points during a trial to prevent the rat from returning to parts of the maze that it had already traversed. White sheets, 20 cm high, were suspended 10 cm from the outside edges of the maze to reduce the use of extramaze cues.

The 12-arm radial maze was described above.

Training

Each rat was food deprived and began training 5 weeks (first replication) or 2 weeks (second replication) after surgery. Each rat was deprived to 85% of its ad-lib weight plus 5 g/week and were fed the appropriate amount after each day's testing. Each rat was handled and then shaped on the wooden runway. During the first day of shaping, food was placed throughout the length of the runway. Each rat was allowed to move freely on the runway for 5 min. During the second day, food was placed only on the far half of the runway, and during the third day food was placed only on the end of the runway.

Testing

Each rat was given 1 trial per day on the Stone maze for 30 consecutive days. At the beginning of each trial, a few pieces of food (Thrive Cat Food, Purina, Inc.) were placed in the goal box, and the rat was placed in the start box. The sliding Plexiglas panel blocking the entrance to the maze was lifted, allowing the rat to enter the maze. The experimenter started a stopwatch when the rat stepped out of the start box. If a rat deviated from the true path to the goal box by placing one of its paws onto a section of the maze leading to a dead end, an error was recorded at that choice point. Retracing errors were not counted; therefore, the maximum number of errors was 14. During the first several trials for each rat, the sliding Plexiglas panels were lowered at six of the choice points to limit retracing after the rat had passed the choice point. When the rat reached the food, the experimenter stopped the stopwatch and recorded the time. The rat was removed from the maze if it did not reach the goal box within 10 min.

Each rat in the second replication of the experiment was also tested on the radial arm maze. At the beginning of each test session, one pellet of food was placed at the end of each arm. The rat was placed in the center of the maze. All the guillotine doors to the arms were raised, and the rat ran down to the end of an arm. All doors except the door to the arm the rat had entered were lowered. The remaining door was lowered after the rat had returned to the center. After the rat was confined to the center for 5 sec, all doors were again raised, allowing the rat to run down an arm. A response was recorded when the rat went more than 25% of the way down an arm. A correct response occurred when the rat went to an arm not previously visited during that test session. An incorrect response occurred when the rat went to an arm previously visited in that test session. This testing procedure continued until all 12 arms had been visited, the rat had made 20 responses, or 10 min had elapsed since the beginning of the test session.

Data Analysis

The number of errors per trial was analyzed with a 3 factor analysis of variance (ANOVA). The 2 between-groups factors were replication (first or second) and experimental group (BF or CON). The 1 within-group factor was blocks of 5 trials (1-6).

The number of errors at each choice point was analyzed by a 3 factor ANOVA. The 2 between-groups factors were again replication (first or second) and experimental group (BF or CON). The 1 within-group factor was the position of the choice point in the maze (1-14).

The data from the radial arm maze were analyzed using a 1-factor ANOVA examining the effect of experimental group on number of errors per test session.

RESULTS

Neurochemistry

ChAT

NBM lesions significantly decreased ChAT levels in the frontal cortex but not in the dorsal hippocampus (see Table 1). In the frontal cortex, the mean decrease in ChAT levels was 66% (compared to the CON values) for rats in the radial maze task, 46% for rats in the T-maze task, 54% for rats in the avoidance tasks, and by 37% in the Stone maze task. Rats were sacrificed 4 months after surgery.

MSA lesions significantly decreased ChAT levels in the hippocampus but not in the frontal cortex. In the hippocampus, the mean decrease was 37% (compared to the CON values) for rats in the radial maze task, 13% for rats in the T-maze task, 24% for rats in the avoidance tasks. and by 31% in the Stone maze task.

Biogenic Amines

Basal forebrain lesions did not significantly alter the levels of endogenous neurotransmitters or their metabolites in the cortex, hippocampus, or caudate nucleus (see Table 2). However, cortical levels of norepinephrine were decreased slightly.

2-Deoxyglucose Uptake

Three weeks following the production of basal forebrain lesions, the level of local cerebral glucose utilization as determined by 2-deoxyglucose uptake did not differ from that in controls.

Serotonin Receptor Binding

The level of ChAT activity in the neocortex decreased significantly, as did the concentration of serotonergic receptors within the NBM. However, there was no significant correlation ($r = 0.32$, $p > 0.05$) between the decrease in ChAT seen in each rat and the associated decrease or change in serotonergic receptor binding. In a previous report we indicated that there was a correlation between these measures. Upon closer examination with more rats, we could no longer identify a significant correlation.

Histology

NBM lesions extended caudally from the anterior commissure within the substantia innominata and were approximately 2 mm in diameter. MSA lesions destroyed most of the cells of the medial septum and the dorsal part of the vertical limb of the diagonal band.

Behavior

Most rats quickly recovered from surgery. Some of the rats with lesions in the NBM, however, showed normal posture but did not eat, drink, or groom. These rats were intubated intragastrically (SMA, Wyeth Lab. Inc., Philadelphia, PA; 10 cc every 6 hr). Rats with lesions in the MSA were generally as healthy (normal grooming, eating, sleeping, etc habits) as the control rats after surgery. At the time of behavioral testing, all rats were healthy and eating normally.

T-Maze Discrimination

Both the NBM and the MSA groups required significantly more trials to reach criterion than the CON group (see Table 3). The mean number of correct responses for the NBM and MSA groups was less than that for the CON group on trials 13-29 and 15-32, respectively, as shown by Scheffe contrasts ($p < 0.01$). All rats eventually reached criterion performance.

Radial Arm Maze Discrimination

The NBM and MSA groups took significantly more trials than the CON group to reach criterion. The choice accuracy of NBM rats was significantly impaired for trials 9 through 20 ($p < 0.05$), and the choice accuracy of MSA rats was significantly impaired for trials 8 through 23 ($p < 0.01$) as shown by Sheffe contrasts. The choice accuracies of the NBM and MSA groups did not differ significantly from each other. All rats eventually reached criterion performance.

Active Avoidance

Acquisition. Both the NBM and the MSA groups required significantly fewer trials than the CON group to reach criterion. The choice accuracies of the NBM and MSA groups did not differ significantly ($p < 0.05$).
Retention. The NBM and the MSA groups did not require significantly more trials than the CON group to reach criterion.

Passive Avoidance

Both the NBM and the MSA groups took significantly more trials to reach criterion. The choice accuracies of the NBM and MSA groups did not differ significantly ($p < 0.05$).

Stone Maze

General. During the first 5 trials, the rats moved slowly

through the maze and took a mean of 4 min, 22 sec to reach the goal. As testing continued, the mean time to reach the goal steadily decreased to a mean of 21 sec.

The rats made many errors at the choice points during the first 5 trials; the mean number of errors for these 5 trials was 6.5. The rats' choice accuracy gradually improved during training; the mean number of errors for the last five trials was 1.8. The effect of trial block number on number of errors per trial was significant ($F = 62.62$, $p < 0.01$). The rats in the first replication made fewer errors per trial than those in the second replication ($F = 9.34$, $p < 0.01$) (see Figure 1).

The choice points differed greatly in their degree of difficulty. Errors were made most often at choices 1, 3 and 11. Choice point position had a significant effect on the number of errors made at each choice point ($F = 89.90$, $p < 0.01$). The rats in the first replication made fewer errors per choice point ($F = 8.56$, $p < 0.01$). An interaction between choice point position and replication number ($F = 2.29$, $p < 0.01$) shows that the rats in each replication had slightly different patterns of errors.

Lesioned Rats. The BF rats performed as well as the CON rats. BF rats made as many errors per trial as the CON rats ($F = 3.90$, $p < 0.05$). The lack of an interaction ($F < 1$) between experimental group and trial block shows that there were no differences in the rate of learning between the two groups (see Figure 1).

The BF rats made slightly fewer errors per choice point than the CON rats ($F = 3.85$, $p < 0.05$). No interaction occurred between experimental group and choice point position ($F < 1$), indicating that lesioned rats and rats with sham operations had the same patterns of errors (see Figure 2).

In the radial arm maze, during the first 5 trials, CON rats made a mean of 8.6 correct choices at the first 12 choice points, whereas BF rats made only 7.3 correct choices. The choice accuracy of CON rats improved rapidly during testing, while that of BF rats improved much more gradually. CON rats made an average of more than 11 correct choices at the first 12 choice points by test session 14, whereas BF rats did not reach this level until test session 21. The CON rats made fewer errors per trial overall than the BF rats ($p < 0.05$) (see Figure 3).

DISCUSSION

Equivalent behavioral changes were obtained from lesions in the NBM and MSA in tasks that varied in their type of motivation, reinforcement, response-reinforcement contingency, and response. Consequently, the behavioral effects of these lesions are not likely to be due to changes in any of the above variables. Rather, they appear to be caused by a fundamental impairment of memory. The pattern of behavioral changes in these tasks is the same as that seen following damage to the hippocampus, a

structure that is important in memory (5). All these data suggest that pathological changes in these two areas of the basal forebrain produce impairments of memory (12). Likewise, both the frontal cortex and the hippocampus, projection areas of the NBM and MSA, respectively, have been closely linked in a system mediating memory (6). The similarity of the behavioral changes resulting from lesions in the NBM and in the MSA suggest that these two projection areas both participate in normal memory functions. The present study suggests that neither the motivation nor responsereinforcement strategy in the memory task is important in terms of the function of the basal forebrain. Performance in a task which differed in motivation (appetitive versus aversive) and response-reinforcement strategy (win-stay versus win-shift) was affected similarly by the lesions. Dissociation of memory is commonly found in amnestic syndromes. Further experiments will be necessary to delineate precisely the types of memory function that are spared and impaired following damage to the basal forebrain areas. Experiments using the Stone maze have shown that performance in tasks requiring trial--dependent memory is consistently impaired following basal forebrain lesions while trial-independent memory, or reference memory, remains intact.

The number of trials to reach criterion asymptotic level of performance determined the level of difficulty fo the different tasks.

The Stone maze is a relatively difficult and complex task. However, the combined lesions of the NBM and MSA produced no impairment of choice accuracy in this task. Control rats took nearly 30 trials to reach a performance level of fewer than 2 errors per trial. Rats with BF lesions reached this level of performance just as rapidly. Furthermore, the pattern of errors at each choice point was identical for the two groups.

The absence of a behavioral effect in the Stone maze was not due to ineffective lesions. Histologically and neurochemically the lesions were very similar to those in previous experiments which did produce behavioral impairments (13). Furthermore, when tested in the radial arm maze in a task which required trial-dependent memory, the same rats exhibited a significant impairment in choice accuracy.

The dissociation shows that the lesions were behaviorally effective, and that the absence of an effect in the Stone maze was due to the type of memory required in the task. The fact that the lesioned rats performed this complex task normally shows that the lack of a behavioral impairment in the simultaneous left/right discrimination in the T-maze was due to the nature of the memory required, rather than to the level of task difficulty.

The rats in replication 1 made fewer errors in the Stone maze than those in replication 2, although this difference was small in magnitude. The reasons for this difference are not clear, but it may have resulted from differences in rats received in separate shipments. This difference does not affect the conclusion of this experiment, however. Within each replication,

the rats with BF lesions performed no worse in the Stone maze than did the control rats. Therefore, the time to initiate training after surgery should not be considered as a behaviorally important factor. These results demonstrate that basal forebrain lesions impair trial-dependent memory but not trial-independent reference memory, and the task difficulty is not the sole factor determining whether basal forebrain lesions produce behavioral impairments.

Our studies on the relationship between the decrease in ChAT activity and the loss of serotonin receptors in the NBM indicated that it is unlikely that serotonergic receptors exist on neurons within the NBM. This conclusion was further supported by other studies conducted in our laboratory. Microinfusion of serotonergic agonists and antagonists into the NBM did not appear to affect cholinergic neuronal activity, as determined by high affinity choline uptake in the frontal cortex (see Appendix). We are presently investigating changes in serotonergic receptor binding in the hippocampus and neocortex following forebrain lesions in the MSA and NBM.

The results of the investigation of changes in brain biogenic amines following NBM lesions are consistent with earlier findings demonstrating that IBO lesions do not alter brain neurochemistry at a distance from the lesion. IBO has been shown to be selectively toxic to cell bodies and does not injure neurons of passage (14). Although the basal forebrain contains catecholaminergic and indolaminergic fiber afferents from the midbrain, the lesions did not have an affect on these systems. Previously reported research has shown that basal forebrain lesions do not alter GABAergic neurons in the cortex or hippocampus (14). Information on possible changes in glutaminergic neuronal systems is at present not available primarily because it is not possible to reliably measure endogenous levels of transmitter-related glutamate in brain tissue. Because glutamate is used by the neuron in greater quantities for non-transmitter purposes, changes in the level of this amino acid are difficult to interpret. We are presently using IBO to investigate the influence of MSA lesions upon levels of endogenous catecholamines and indolamines and their metabolites in the hippocampus.

Recent reports have shown that 2-deoxyglucose utilization is decreased throughout the brain following basal forebrain lesions. Glucose utilization recovers to normal levels within 3 weeks after the production of these lesions. The present study confirmed these findings. The return to control levels of cerebral glucose utilization parallels a similar return to normal levels in sodium-dependent high affinity choline uptake in the cortex following basal forebrain lesions. Compensatory changes in postsynaptic muscarinic receptor density profiles also appear and have been documented 3 weeks following the production of basal forebrain lesions. The possible correlation between these events and the recovery of behavior which is seen in rats with basal forebrain lesions will be investigated in future studies.

These results on the natural recovery abilities of the young rat brain may have importance for our studies on recovery of function. An understanding of how lesioned rats are able to recover from biochemical abnormalities in the brain and to regain the ability to perform difficult behavioral tasks may be useful in designing therapies to produce a recovery of function in man.

Table 1. Choline acetyltransferase levels (mean ± S.E.M) in frontal cortex and dorsal hippocampus of rats in each of the three behavioral studies.

GROUP	N	CHOLINE ACETYLTRANSFERASE (nmol/mg protein/h) FRONTAL CORTEX	HIPPOCAMPUS
T-MAZE			
Control	6	22.23 ± 0.91	27.27 ± 2.88
NBM	6	15.69 ± 1.34*	29.10 ± 2.24
MSA	7	20.39 ± 1.81	17.90 ± 1.77**
RADIAL ARM MAZE			
Control	9	23.00 ± 1.31	26.25 ± 0.77
NBM	7	11.61 ± 2.04**	33.72 ± 1.03
MSA	10	21.58 ± 1.57	13.56 ± 2.38**
AVOIDANCE			
Control	9	24.90 ± 1.16	35.67 ± 1.00
NBM	9	19.13 ± 1.89*	41.89 ± 3.63
MSA	9	24.26 ± 1.07	18.62 ± 2.49**

*$p < 0.05$, **$p < 0.01$ compared to corresponding region of control rats using Student's 2-tailed test. NBM lesions significantly decreased ChAT activity in cortex but not in hippocampus, while MSA lesions significantly decreased ChAT activity in hippocampus but not in cortex. The number of rats in each group is indicated.

Table 2. Changes in biogenic amine and metabolite levels in three brain regions of rats with NBM lesions.

	PERCENT CHANGE FROM CONTROLS					
REGION	N	DOPAC	DA	5HIAA	HVA	5HT
CORTEX	-23	NC	NC	NC	NC	NC
HIPPO	-17	NC	+16	NC	NC	NC
CAUDATE	NC	-22	NC	NC	NC	-10

Control rats did not receive IBO injections. N = norepinephrine, DOPAC = 3,4-dihydroxyphenylacetic acid, DA = dopamine, 5HIAA = 5-hydroxyindole acetic acid, HVA = homovanillic acid, 5HT = serotonin. N = 10. NC indicates no change greater than 5% from control levels.

Table 3. Mean number of trials to criterion for each behavioral task.

TASK	TRIALS TO CRITERION		
	CON	NBM	MSA
	X ± S.E.M.		
T-Maze	23.7 ± 2.4	35.1 ± 3.1 **	36.9 ± 2.7 **
Radial Maze	18.0 ± 0.8	27.1 ± 2.4 *	28.8 ± 1.89 **
Active Avoidance:			
Acquisition	25.5 ± 1.3	20.1 ± 1.5 *	18.9 ± 2.3 *
Retention	16.4 ± 0.7	17.1 ± 0.9	16.2 ± 1.3
Passive Avoidance	1.9 ± 0.3	4.6 ± 0.9 *	4.5 ± 0.8 *

* p 0.05 vs. CON group of rats, ** p 0.01. The results are the mean (± S.E.M.) for 7-10 rats in each behavioral task. CON = sham-operated controls; NBM = lesions in the nucleus basalis magnocellularis; MSA = lesions in the medial septal area.

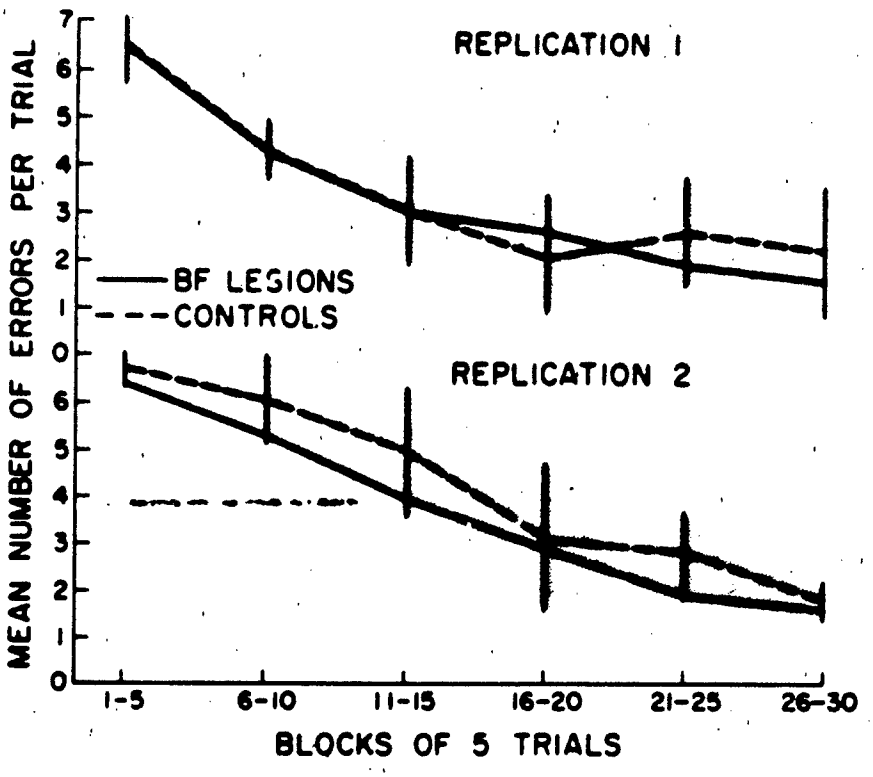

Figure 1. The mean number of errors per trial in the Stone maze as a function of trial number. The stippled area represents ± 1 standard deviation (S.D.) from the mean of the CON rats. Errors per trial decreased for both groups at a similar rate.

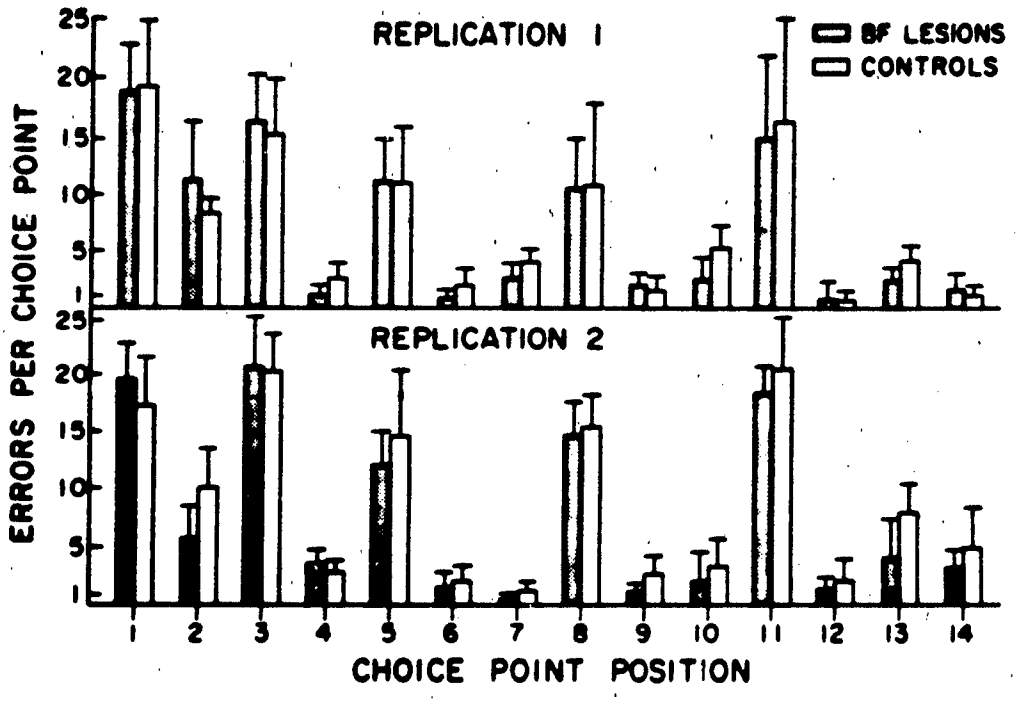

Figure 2. The mean number of errors (± S.D.) on each choice point in the Stone maze as a function of choice point position. The BF rats made slightly fewer errors per choice point than did the CON rats. There was no interaction between experimental group and choice point position.

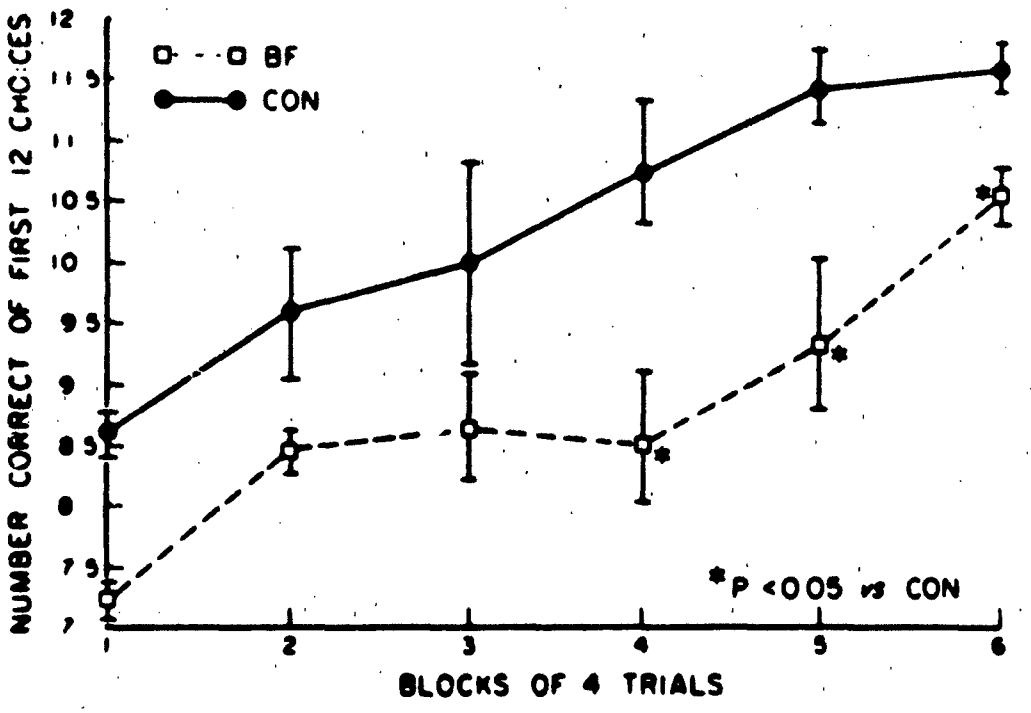

Figure 3. The mean number of correct choices in the first 12 choices (±SEM) as a function of trial number. CON rats made significantly ($p<0.05$) fewer errors than did BF rats.

FIGURE CAPTIONS

Figure 1. The mean number of errors per trial in the Stone maze as a function of trial number. The stippled area represents ± 1 standard deviation (S.D.) from the mean of the CON rats. Errors per trial decreased for both groups at a similar rate.

Figure 2. The mean number of errors (± S.D.) on each choice point in the Stone maze as a function of choice point position. The BF rats made slightly fewer errors per choice point than did the CON rats. There was no interaction between experimental group and choice point position.

Figure 3. The mean number of correct choices in the first 12 choices and standard errors of the mean as a function of trial number. CON rats made significantly ($p < 0.05$) fewer errors than did BF rats.

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APPENDIX

PHARMACOLOGICAL MANIPULATIONS OF THE SUBSTANTIA INNOMINATA-CORTICAL CHOLINERGIC PATHWAY

The major source of the neocortical cholinergic (Ch) innervation is from the substantia innominata (SI). Although the neurochemical and histological features of the cells in the SI have been thoroughly investigated, little is known concerning its regulatory afferent inputs. The present study examined the effects of various pharmacological agents injected into the SI in order to determine the transmitter systems that influence activity of basal forebrain Ch cells. Sodiumdependent high affinity choline uptake (SDHACU) in the frontal neocortex, a projection field of the SI, was the measure of Ch neuronal activity. In vitro SDHACU is a rapid and reliable measure of the activity of cholinergic neurons in vivo.

Male Sprague-Dawley rats (Charles River, 250-300 g) had 22 gauge cannulae chronically implanted for local microinjection into the SI. All infusions of the pharmacological agents were made unilaterally into unanesthetized, freely moving rats. An internal cannula (28 gauge, Plastic Products Inc.) was securely fastened to the guide cannula prior to the microinfusion. The internal cannula was attached to a 1 ul Hamilton syringe by a 45 cm flexible Teflon tubing which was prefilled with the appropriate agent prior to insertion. The pharmacological agents were prepared immediately prior to infusion in isotonic phosphate-buffered saline, final pH 7.4. The exogenous agents were chosen in order to delay the normally rapid metabolic processes that inactivate endogenous transmitter substances. The agents were injected rapidly during 1 min to achieve a broad dispersion throughout the SI. The injection volume was 1 ul. The internal cannula remained in position for 1 minute. The rats were then returned to their home cages. Controls received buffered saline injections.

An appropriate time to wait in order to achieve maximal changes in neocortical SDHACU following a pharmacological or behavioral manipulation is 50 min. The rats were sacrificed by decapitation. The brain was quickly removed and rinsed with ice-cold 0.9% saline, and rapidly cooled on ice. The right and left frontolateral cerebral cortex (approx. 50 mg ea.) were removed and quickly homogenized in 20 vol of ice-cold 0.32 M sucrose. The area sampled included prefrontal and premotor cortex but not cingulate cortex or the anterior dorsal ridge of the rhinal sulcus. The time from death to homogenization was less than 2 min. SDHACU was determined according to a modified method of Simon et al., (1). Aliquots of the synaptosomal suspension were incubated for 4 min in the presence of (^{3}H)-choline (80 Ci/mmol, New England Nuclear, Boston, MA, final conc: 0.4 uM). Uptake was terminated by the addition of 2 ml of ice-cold sodium-free Tris Krebbs Ringer solution which was

followed immediately by rapid filtration on Whatman GF/C glass filters using a Brandel Cell Harvester (Biomedical Research and Development Laboratories, Gaithersburg, MD). The filters were washed three times with 5 ml of ice-cold buffer and then placed in liquid scintillation vials. Ten ml of Aquasol II (New England Nuclear) were added, and the radioactivity counted in a Packard Liquid Scintillation Spectrometer (Model 4640). Protein was measured in every sample (2), with bovine serum albumin as external standard. Statistical comparisons were made by a Student's t-test for each substance.

Muscimol, enkephalin and imipramine significantly ($p < 0.05$) decreased cortical SDHACU (See Table 1). Glutamate significantly ($p < 0.05$) increased cortical SDHACU. The other pharmacological agents produced no significant changes.

The present study identifies some of the afferent systems that synapse on cells in the SI. GABAergic and enkephalinergic systems have an inhibitory influence, glutamatergic systems have an excitatory input, while adrenergic, dopaminergic and serotoninergic neurotransmitter systems apparently have no influence.

The administration of neurotransmitter receptor agonists directly into the brain restricts the probable locus of action of these drugs more than systemic administration. The effects of such local infusion into the SI thus more faithfully reflect the possible interactions of afferents to the SI with Ch neurons. GABA, glutamate, or enkephalin containing neurons may be regulating Ch activity in the SI either directly or via interneurons. They may also presynaptically modulate other neuronal afferents into the SI. Either conclusion would be consistent with the results of the present study. The lack of an effect by an agent may be because an insufficient amount was available to affect Ch neuronal activity. The dose of (±)-2-amino-5,6-dihydroxy-1,2,3,4-tetrahydronaphthalene (ADTN) was limited by its solubility in the buffer. Otherwise the doses chosen were as high as possible as long as the infusate was isotonic and the pH could be adjusted appropriately. If an agent did not alter SDHACU, another agent known to affect a similar class of receptors was also tested. For example, two adrenergic and two serotonergic agonists were examined. The antagonists naloxone and picrotoxin were injected (i.p.) prior to enkephalin and muscimol, respectively, in order to confirm the specificity of action of the agents within the SI.

The changes in SDHACU reported in the present study are consistent with the results of earlier anatomical and pharmacological studies. Immunocytochemical investigations have found axosomatic and axodendritic synapses containing glutamic acid decarboxylase, the synthetic enzyme for GABA, in the SI. These putative GABAergic terminals may originate in the nucleus accumbens. Evidence for the presence of GABAergic receptors within the SI is supported by studies showing that the microinfusion of muscimol into the SI decreased cortical acetylcholine turnover. The change in acetylcholine turnover was reversed by

prior picrotoxin injection.

The present study also substantiates earlier reports on the presence of enkephalinergic and glutamatergic terminals and receptors within the SI. The reduction in SDHACU reported in the present study is consistent with earlier reports that opiate injections into the ventricles inhibit acetylcholine release.

This study identifies some of the afferent systems that influence activity in the SI. The demonstration of regulatory inputs to the SI is of importance for designing effective therapies for patients with destruction or degeneration within this brain region.

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Table 1. The Effects of Various Pharmacological Agents Upon Cholinergic Activity in the Substantia Innominata

AGENT (n)	PHARMACOLOGICAL EFFECT	AMOUNT INJECTED+	MEAN PERCENT CHANGE
		nmole	
Decrease SDHACU			
Muscimol (8)	GABAergic Agonist	44	-25*
Muscimol (5)	GABAergic Agonist	88	-50*
Enkephalin (6)	Opiate Agonist	40	-40*
Imipramine (4)	Reuptake Inhibitor++	100	-47*
Increases SDHACU			
Glutamate (6)	Excitatory A.A.	20	+25*
Does Not Affect SDHACU			
Saline (10)	Isotonic, pH 7.4		
+10			
LSD (4)	Serotonergic Agonist	10	+1
Serotonin (4)	Serotonergic Agonist	1000	+2
Clonidine (4)	Adrenergic Agonist	10	+2
Isoproterenol (6)	Adrenergic Agonist	100	+14
Muscimol (4)	GABAergic Agonist	11	+5
ADTN (4)	Dopaminergic Agonist	100	+9
Enkephalin Naloxone** (4)	Agonist + Antagonist	40	+14
Muscimol Picrotoxin# (4)	Agonist + Antagonist	44	+3

* p 0.05 by t-test.
**Given 10 min. prior to enkephalin infusion (5 mg/kg, i.p.)
+Total injection volume was 1.0 ul.
++Imipramine is a norepinephrine and GABA re-uptake inhibitor.
#Given 5 min. prior to muscimol infusion (4.4 mg/kg, i.p.)

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